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ANTIDIABETIC STUDIES OF THE CRUDE METHANOLIC EXTRACTS OF LEAVES AND FLOWERS OF *BUTEA MONOSPERMA*, (LAUM.), A MEDICINAL PLANT, USING WISTAR RATS

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Abstract

The present study investigates the antidiabetic potential of *Butea monosperma leaves and flowers* on biochemical profile in alloxan-induced [200mg/kg] diabetic rats. The effects of methanolic fraction of *Butea monosperma* on body weight, blood glucose, total protein, serum creatinine and blood urea were examined in control and experimental groups of animals. Rats with fasting plasma glucose (FPG) range of 280–350 mg/dl were considered diabetic and included in the study. 15th day results showed significant increase in fasting plasma glucose levels in STZ-control ($p<0.01$) rats when compared with normal control group of animals, and same was significantly reversed by methanolic extracts treated and Glibenclamide treated groups. The antihyperglycemic action of methanolic active fraction of flowers results from the potentiation of insulin release from existing beta cells of the islets of Langerhans. By this it was confirmed that methanolic active fraction of *B.monosperma* leaves showed effective result in anti diabetic activities in a safe manner at the single dose 50 mg/kg b.wt than that of the flower extract Body weight was significantly decreased in the diabetic control group when compared with normal group. Oral administration of methanolic active fractions of B.*monosperma* leaves and flowers for 45 days significantly increased ($P<0.05$) the body weight in diabetic groups, respectively among which leaf extract showed better result. During the present study the different doses of active fractions of B.monosperma leaves and flowers, did not exert any toxic effect and it can be concluded that *B.monosperma* active fractions are not lethal in the usual range of oral anti-diabetic drug i.e. 50mg to 2000mg/kg b.wt in experimental animal models. The 100 mg/kg b.wt dose of active fraction of *B.monosperma* flowers and 50 mg/kg b.wt of active fraction of *B.monosperma* leaves are considered to be safe.

Keywords: *Butea monosperma*, Antidiabetic activity, Oral Glucose Tolerance test, Methanol extract.

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Introduction

The use of medicinal herbs for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. Medicinal herbs are significant source of pharmaceutical drugs. Latest trends have shown increasing demand of phytodrugs. Medicinal herbs and extracts prepared from them are widely used in the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite. Medicinal herb is a biosynthetic laboratory, for chemical compounds like glycosides, alkaloids, flavonoids, bianthra-

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quinones, resins, and oleoresins, etc. one such important medicinal plant is Butea monosperma. Though in the tribal areas and in the village's people claim that the medicinal plants are efficacious, scientific validation is wanting. There is an urgent need for the scientific experimental study. In this research study an attempt has been made to scientifically prove the anti-diabetic potential of this plant.

Chemical constituents

Flower – Triterpene ,several flavonoids butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, onospermoside(butein 3-e-D-glucoside) and isomonospermoside, chalcones, aurones, isobutyine, palasitrin, 3',4',7- trihydroxy - flavone. Myricyl alcohol, stearic, palmitic, arachidic and lignoceric acids glucose, fructose, histidine, aspartic acid, alanine and phenylalanin Leaves - Glucoside, Kino-oil containing oleic and linoleic acid, palmitic and lignoceric acid.

Materials and methods

Collection of animals

Male Wistar rats weighing 110-150g were obtained from National Institute of Nutrition, Hyderabad, India. They were acclimatized to laboratory conditions for a week prior to the initiation of the experiment, four groups of animals (each group contain 6 animals) were kept in each cage (45 x 30 cm). The cages were maintained in a clean and hygienic condition and the animals were fed with a standard laboratory diet (Godrej Agro Food Industries, Bangalore, India) and tap water. Twelve hours before the start of the experiment, rats were deprived of food, but given free access to water.

Preparation of leaf extract

The whole plant will be shade dried and subjected to size reduction to get a coarse powder. The powdered material will be subjected to successive extraction in a Soxhlet apparatus, using methanol (90%) as solvent at 50°. The extract will be then evaporated on a rotary evaporator. The same procedures are followed to the extraction of Methanol and Ethyl acetate.

Experimental induction of diabetes

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared Streptozotocin (STZ) (30 mg kg-1 b.w.) in 0.1 M citrate buffer (pH – 4.5) in a volume of 1 ml /kg b.w. Rats were supplied with 5% glucose solution for 48 h after STZ injection in order to prevent hypoglycemia. The control animals were treated with citrate buffer (pH – 4.5). Diabetes was developed and stabilized in these STZ treated rats over a period of 7 days. The control animals were treated with citrate buffer (pH-4.5). After 7 days of STZ administration, plasma glucose levels of each rat were determined. Rats with fasting plasma glucose (FPG) range of 280–350 mg/dl were considered diabetic and included in the study. Blood was collected by sinocular puncture.

Effect of *Butea monsperma* on Fasting Plasma Glucose and plasma insulin levels in STZ-induced diabetic rats- 15 day study: Rats were divided in to 7 groups of 6 rats each. Group 1 normal control rats received vehicle alone (Dimethylsulfoxide [DMSO] 0.5%; 1ml/kg b.w.). Group 2, 3 normal rats received orally 100 and 200 mg/kg b.w. of *Butea monsperma* . Group 4 diabetic control rats received vehicle alone. Group 5 & 6 diabetic rats received orally 100 and 200 mg/kg. b.w. of ethyl acetate and methanol extracts respectively. Group 7 diabetic rats received commercial drug Glibenclamide. Blood samples were collected periodically in all the experimental groups.

Result

Experimental design 1

Group 1 – normal rats treated with vehicle alone

Group 2 – streptozotocin induced diabetic rats treated with vehicle alone

Group 3- Streptozotocin induced diabetic rats+ butea monosperma leaf extract (100 mg/kg)

Group 4- STZ induced diabetic rats + B.M flower extract (100 mg/kg)

Group 5 -STZ induced diabetic rats + Glibenclamide (600 µg/kg bw)

Values are expressed in Mean±S.D

Effect of *Butea monosperma* on fasting blood glucose in normal and stz-diabetic rats.

	Group I	Group II	Group III	Group IV	Group V
Day 1	90±6.05	93.6±3.90a	413.5±5.79	452.5±6.99b	448.5±4.71b
Day 15	91±6.13	92.16±2.91a	410.3±7.3	332.5±6.9b	278.16±7.42:
Day 30	91.5±5.79	90.5±6.8a	389.8±5.39	157.3±7.11a	141.5±7.95a
Day 45	91.83±8.27	92.6±4.81a	366±7.23	138.6±4.71a	131.5±6.02a

Each value is mean ± SD for 6 rats in each group .a: p<0.05 by comparison with normal rats.
b: p<0.05 by comparison with streptozotocin diabetic rats.- : No significance

Biochemical Parameters

Statistical analysis

All values are expressed as mean ± SEM, statistical significance was analysed using one way ANNOVA followed by Turkey-Krammer multiple comparison test**.** The data were considered significant at *P<0.05*

Study design- 3 report

Body weight

Initial & final body weights of all the animals were observed normal and values were shown in table 1. Body weight was significantly decreased in the diabetic control group when compared with normal group. Oral administration of methanolic active fractions of B.*monosperma* leaves and flowers for 45 days significantly increased (P<0.05) the body weight in diabetic groups, respectively among which leaf extract showed better result. (Ref: Table 1 and Graphs)

Table No. 01: Effect of *B.monosperma* on body weight in control and STZ-diabetic rats.

Body Weight	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Initial Body Weight (g/100 g) (Day 1)	165.61±2.18	165.21±4.15	164.74±3.33	164.74±3.35	166.74±5.67	166.76±3.68	167.08±3.48
Final Body Weight (g/100 g) (Day 45)	164.22±4.26	160.27±5.44	158.25±5.41	143.67±1.53	150.83±2.11	151.88±6.99	154.34±5.35

Each value is mean ± SD for 6 rats in each group.

Table No. 02: Effect of *B.monosperma* on oral glucose tolerance in control and STZ-diabetic rats.

Blood glucose (mg/dl) Time (min) after glucose administration							
Blood Glucose	**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**	**Group VII**
Initial	70.20±1.59	70.79±3.90	71.46±3.67	70.71±2.73	71.03±3.82	69.83±1.45	70.31±1.32
1h	70.30±1.23	72.53±3.10	71.94±2.99	144.46±3.85a	b121.60±1.66	b103.88±4.39	ab 95.07±3.23
2h	70.56±2.71	71.70±2.86	71.30±2.29	136.42±4.25 a	b101.35±4.45	b92.21±3.54	a b 82.09±3.46
3h	71.47±3.64	72.18±3.17	72.67±2.13	131.96±4.09	b95.99±4.90	b81.69±4.96	a b77.36±4.27

Each value is mean ± SD for 6 rats in each group.*p*<0.05 by comparison with normal rats.

a. *p*<0.05 by comparison with STZ- diabetic rats.

Table No. 03: Effect of *B.monosperma* on serum and tissue total Hexose levels in control and STZ-diabetic rats.

Levels	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Plasma (mg/dL)	101.82±0.97	99.01±2.78	100.59±2.01	136.79±3.89	120.79±3.65	111.94±3.01	105.50±4.81
Liver (mg/100 g)	36.23±4.67	35.50±3.05	65.20±4.39	b44.14±1.99	b39.56±1.04	ab36.82±2.311	
Kidney (mg/100 g)	28.78±2.04	28.68±2.68	26.31±3.08	54.14±22.44	b41.31±2.64	b37.59±2.02	ab34.59±3.63

Table No. 04: Effect *B.monosperma* on serum total cholesterol, triglycerides and free fatty acid levels in control and STZ- diabetic rats.

Total	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Cholesterol (mg/dl)	93.70±3.13	99.63±1.11	98.29±4.09	212.08±3.39	b133.88±1.38	b102.2±1.38	ab112.35±1.73
Triglyceride (mg/dl)	16.45±1.06	17.53±1.85	17.29±1.1.41	47.76±24.62	b24.62±2.23	b22.31±3.14	ab21.19±2.99
Free fatty (mg/dl)	70.23± 2.67	75.71±3.96	74.72±4.14	135.91±3.82	b108.59±4.33	b86.50±2.79	ab82.55±2.88

Each value is mean ± SD for 6 rats in each group.

a: p<0.05 by comparison with normal rats.p<0.05 by comparison with STZ- diabetic rats. - : No significance.

a- µmol of pyruvate liberated per hour b-

b: µmol of phenol liberated per minute c- µmol of p-nitroanilide liberated per minute.

Table No. 05: Effect of *B.monosperma* on serum HDL, LDL and VLDL levels in control and STZ-diabetic rats.

Group	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
HDL- Cholesterol (mg/dL)	51.76±3.5	50.91±2.72	51.53±2.29	a26.60±1.75	b37.42±2.37	b39.94±1.07	ab45.54±4.06
LDL- Cholesterol (mg/dL)	30.76±1.4	34.09±1.49	80.85±1.09	a51.90±2.28	b38.39±3.19	b35.86±4.02	ab34.85±2.69
VLDL-Cholesterol (mg/dL)	18.56±1.89	20.37±2.07	20.84±2.06	a38.46±3.43	b25.50±3.16	b23.08±2.63	ab21.57±3.51

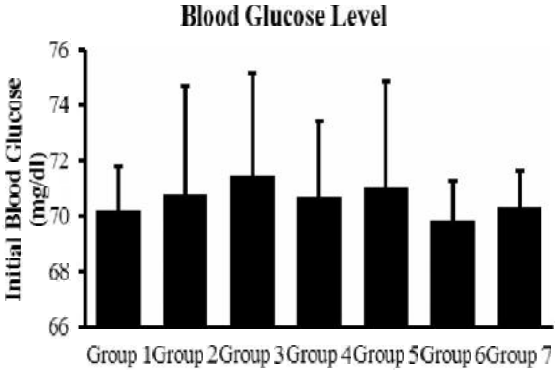
Each value is mean ± SD for 6 rats in each group. $p<0.05$ by comparison with normal rats.

a. $p<0.05$ by comparison with STZ- diabetic rats. - : No significance.a- µmol of pyruvate liberated per hour;

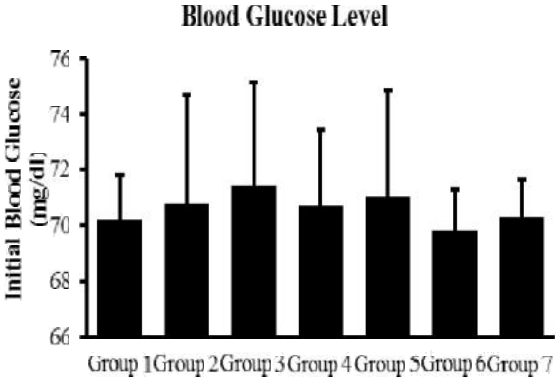
b. µmol of phenol liberated per minute; c- µmol of p-nitroanilide liberated per minute.

Histopathological examination

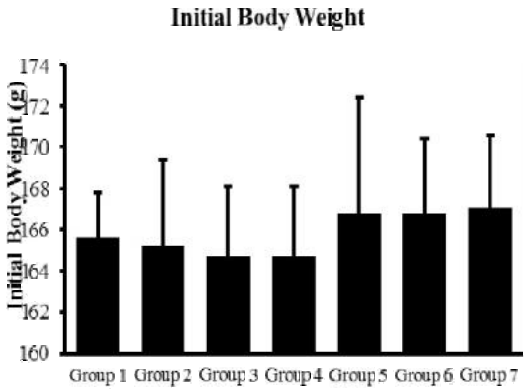
The animals were sacrificed after each experiment, liver, kidney and spleen were dissected and rinsed in physiological saline water and fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and processed for paraffin embedding. Sections of 5µm thickness were taken using a microtome. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin and subjected to histopathological examination



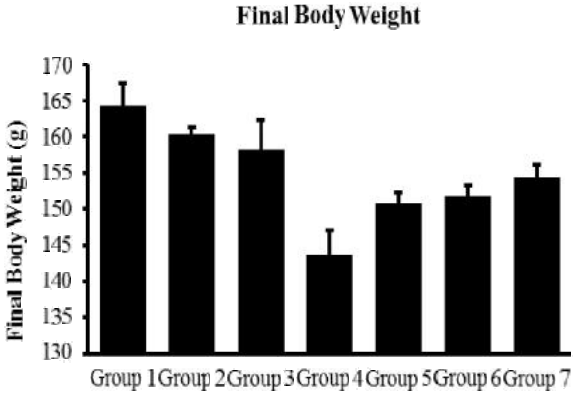
Graph: 1A: Initial blood glucose level



Graph: 1B: Blood glucose level after 1hr



Graph: 1C: Initial Body Weight



Graph: 1D: Final Body Weight

Discussion

Antihyperglycemic effect of methanolic active fractions of B.Monosperma leaves and flowers

The increased level of FPG in STZ-diabetic rats was lowered by oral administration of methanolic active fraction of B.monosperma leaves and flowers for 45 days. The antihyperglycemic action of methanolic active fraction of B.monosperma flowers results from the potentiation of insulin release from existing beta cells of the islets of Langerhans In streptozotocin induced diabetic rats, the levels of cholesterol, free fatty acid and triglycerides are elevated in liver, kidney, heart and brain. Similarly, literature reported the increased levels of tissue lipids in STZ-induced diabetic rats.

In our study, oral administration of methanolic active fractions of B.monosperma leaves, flowers and glibenclamide significantly ($p < 0.05$) decreased the tissue lipid (TC, TG and FFA) levels. The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acid synthesis. Also it may be due to improving the level of insulin secretion. Literature has shown flavonoids, alkaloids and terpenoids to be the active hypoglycemic principle in many medicinal plants with blood glucose and lipids-lowering attributes reported that the presence of alkaloids in the plant aqueous extract, may account for the observed hypoglycemic and hypolipidemic effects. Significant lowering of total cholesterol and raise in HDL cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischemic conditions.

Conclusion

During the present study the different doses of active fractions of B.monosperma leaves and flowers, did not exert any toxic effect during the present study. From the present study, it can be concluded that *B.monosperma* active fractions are not lethal in the usual range of oral anti-diabetic drug i.e. 50mg to 2000mg/kg b.wt in experimental animal models. The 100 mg/kg b.wt dose of active fraction of *B.monosperma* flowers and 50 mg/kg b.wt of active fraction of *B.monosperma* leaves are considered to be safe which is confirmed by our observation.

Another important objective of this study was the anti-inflammatory activity of methanolic active fraction of Butea monosperma leaves. It was proved that the three different doses methanolic active fractions of Butea monosperma leaves (50, 100, 250 mg/kg b.wt) showed significant dose dependent anti-inflammatory activity on FCA induced inflammation in Wistar rats by normalizing the paw volume, paw thickness and body weight. The results were comparable with the reference drug Prednisolone (10 mg/kg P.O).

By this it was confirmed that methanolic active fraction of B.monosperma leaves showed effective result in anti diabetic activities in a safe manner at the single dose 50 mg/kg b.wt than that of the flower extract and this leaf extract had a promising result with anti-inflammatory activity.

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